Indonesian Journal of Biotechnology, December, 2010 Vol. 15, No. 2, pp. 86-92

Ethanol Production by Fermentation of Various Sweet-stalk Sorghum Juices Using Various Yeast Strains

Donny Widianto[1]*, Akbar Arofatullah[1], Triwibowo Yuwono[1], and Irfan Dwidya Prijambada[1]

[1]Department of Agricultural Microbiology, Faculty of Agriculture, Gadjah Mada University, Yogyakarta, Indonesia

Abstract

The ethanol production by fermentation of sweet-stalk sorghum juice is affected by the juice composition and the capability of the yeast strain to ferment it. Eight yeast strains were tested on their growth and ethanol fermentation abilities in sweet-stalk sorghum juices extracted from three cultivars of sweet sorghum. The best specific growth rate of the yeast strains grown aerobically in the yeast extract peptone dextrose (YEPD) broth and the sweet-stalk sorghum juices of KCS105, FS501, and FS902 cultivars, were achieved by OUT7903, OUT7913, OUT7903, and OUT7027 yeast strains, respectively. However, the best specific CO_2 evolution rate of the yeast strain during fermentation of the juices was achieved by OUT7027 yeast strains. The highest ethanol concentration, ethanol yield, and sugar conversion efficiency (SCE) were obtained by strain OUT7921 when it was employed to ferment sweet-stem sorghum juice of FS902 cultivar. It was also observed that the juice extracted from sweet-stalk sorghum of FS902 cultivar is the most suitable medium for all yeast strains to achieve their best fermentation abilities. Thus, it is likely that the growth and ethanol production ability of a yeast strain in sweet-stalk sorghum juice depend on the physiological responses of the yeasts to nutrient composition of the sorghum juice and the sorghum cultivar from which the juice was extracted.

Keywords : Sweet-stalk sorghum juice, ethanol, fermentation, yeast

Introduction

The increase consumption of fossil-fuels and enhanced greenhouse effects had made renewable resources being considered as an alternative for overcoming the shortage of fossil energy and controlling the atmosphere concentration of CO2. Because of these concerns, initiatives have been made to develop cleaner, more reliable fuels that will reduce the dependence on fossil fuels. Currently, ethanol has emerged as one of the most viable options in the area of non-conventional sources of energy (Gnansounou & Dauriat, 2005; Saxena *et al.*, 2009)

Sweet sorghum is an attractive feedstock for ethanol production because of its high fermentable sugars, high yield of green biomass (20–30 dry tons/ha), low requirement for fertilizer, high efficiency on water usage (1/3 of sugarcane and 1/2 of corn), short growth period (120–150 days), and its' adaptability to diverse climate and soil (Prasad *et al.*, 2007; Rooney *et al.*, 2007; Steduto *et al.*, 1997; Tsuchihashi and Goto, 2005; Tsuchihashi and Goto, 2004). In Indonesia, sweet sorghum was firstly introduced in the 1980s and was studied by the

* **corresponding author :**
Donny Widianto,
Department of Agricultural Microbiology, Faculty of Agriculture, Gadjah Mada University, Jl. Flora Bulaksumur, Yogyakarta, Indonesia, 55281
E-mail : donny@faperta.ugm.ac.id

Indonesian Sugar Research Institute as a raw material for sugar (Sumantri and Purnomo, 1997). Until now, little was known about the usage of sweet sorghum as a feedstock for ethanol production by fermentation in Indonesia.

The ability of yeast to produce ethanol from sweet-stalk sorghum juice depends on many factors such as fermentation techniques, strains, sugar profile (amounts of sucrose, glucose and fructose), and the existence of inhibitory substances. Fed-batch fermentation was previously reported had a higher conversion efficiency than batch fermentation (Laopaiboon *et al.*, 2007), and application of immobilized yeast in a fluidized bed reactor not only shortened fermentation time significantly but also increased conversion efficiency (Liu *et al.*,2008). Day and Sarkar (1982) reported that ethanol productivity varied significantly among different yeast strains and ethanol yields differed among juice batches. Imam and Capareda (2010) concluded that ethanol production and fermentation efficiency varied depending on the sweet sorghum crop as well as the amount and proportion of sugar content. Another research group (De Mancilha *et al.*, 1984) found out that yeast strains which fermented molasses efficiently, did not necessarily work best on sweet sorghum juice. Gibbons and Westby (1989) noted yeast inhibition by some factor(s) present in juice from sweet sorghum cultivar NK 8368 but was not observed with cultivar NK 405.

This study was conducted for the purpose of evaluating and selecting yeast strains for their ability to produce ethanol using sweet-stalk sorghum juices as the substrates.

Materials and Methods

Yeast strains

Eight yeast strains, *Saccharomyces awamori* (OUT7009), *Saccharomyces mandshuricus* (OUT7027), *Saccharomyces italicus / Saccharomyces steineri* (OUT7913, OUT7921, and OUT7903), *Saccharomyces chevalieri* (OUT7096), *Saccharomyces sp.* (OUT7055), and *Saccharomyces ellipsoideus* (OUT7080), obtained from the culture collection of Laboratory of Molecular Genetics, Graduate School of Engineering, Osaka University, Japan, were used. The strains were maintained on yeast extract peptone dextrose (YEPD) agar plates at 4°C and subcultured every four weeks.

Sweet-stalk sorghum juices

Three sweet sorghum cultivars, FS501, FS902, and KCS105 (Nitta, *et al.*, 2008), obtained from the College of Agriculture, Ibaraki University, Japan, were grown in December at a location (Kalasan) in Yogyakarta. Stalks were harvested in late May and pressed after heads and leaves were removed. Juices were autoclaved and stored in a refrigerator (4°C) immediately after harvest. The sugar concentration of the juice was measured using a portable refractometer (ASONE, Spittz IPR-101α), and the sugar profile was analyzed by High Pressure Liquid Chromatography (HPLC) of Beckman156 HPLC system (Beckman Instruments, Palo Alto, USA) using an Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA) at 45 °C. The eluent, 0.048N H_2SO_4, was used at a flow rate of 0.5 ml/min.

Cultivation and fermentation media

Yeast extract peptone dextrose (YEPD) broth was used only for propagating yeast cells aerobically. The sweet-stalk sorghum juice containing sugar concentration of 10 °Bx and supplemented with KH_2PO_4 (0,45 g l^{-1}) and $(NH_4)_2SO_4$ (4,6 g l^{-1}) was also used for propagating yeast cells aerobically as well as for fermentation medium.

Yeast cultivation

Overnight culture of yeast cells were inoculated into 100 ml YEPD broth or

supplemented sweet-stalk sorghum juice containing sugar concentration of 10 ^{o}Bx in 500 ml erlenmeyer flask to give final concentration of 10^4 cells ml^{-1} and incubated on rotary shaker at ambient temperature. The cell growth was monitored at appropriate intervals by observing their optical density $(OD)_{600}$ nm value using a spectrophotometer (Spectronic 21D, Milton Roy). Specific growth rate (m) of cells was calculated using the standard equation method (Monod, 1949).

Ethanol fermentation

The ethanol production was carried out in a batch fermentation. An exponentially growing culture of each yeast strains was inoculated into 1 l fermentation medium in a 2 l glass jar to give a final concentration of 10^6 cells m l^{-1}. The glass jar was closed with a rubber stopper equipped with glass and plastic tube for measuring CO_2 evolution and incubated statically at ambient temperature. Ethanol concentration was determined using modified Conway micro-diffusion method (Kaye, 1980). The CO_2 evolution during fermentation was monitored periodically by measuring the volume of produced gases (Sato and Yoshikawa, 1988). The sugar conversion efficiency (SCE) which expressing the ability of yeasts to produce ethanol from the available sugars was calculated using equation below as described by De Mancilha *et al.* (1984).

$$SCE = \frac{\text{alcohol content (\%w/v) x 100}}{\text{media sugar content (\%) x 0.504}}$$

Results and Discussion

Sweet-stalk sorghum juices sugar profile

Sugar content and profile in sweet sorghum juice of different cultivars can be different (Prasad *et al.*, 2007). Fermentable sugars in sweet sorghum are mainly sucrose, glucose, and fructose. The sweet-stalk sorghum juices sugar profile of three cultivars, KCS105, FS501, and FS902, is presented in Table 1.

Table 1. Sugar profile of sweet stalk sorghum juice

Sweet Sorghum Cultivars	Concentration (%)		
	Sucrose	Glucose	Fructose
KCS105	3.559	2.061	2.171
FS501	2.084	2.818	3.685
FS902	3.158	2.444	2.633

The sugar profile of FS501 juice was different from KCS105 and FS902 juices in which it contained more glucose and fructose than sucrose. Similar sugar profile was observed in KCS105 and FS902 juices, with levels of glucose and fructose contents in FS902 juice was higher than KCS105. The sugar profile differences among the juices might affect the growth and fermentation ability of the yeast strains when it used as cultivation or fermentation medium. Previous research showed that common ethanol fermentation yeasts, strains of *Saccharomyces cerevisiae*, utilized sugars in mixtures of fermentable sugars in a certain order with most ethanol producing yeasts utilized sugars in the order of sucrose, glucose, and fructose (Berthels *et al.*, 2004; Meneses *et al.*, 2002). Thus, sucrose and glucose are always first consumed and converted into ethanol before fructose in a condition if a feedstock with mixed sugars like sweet sorghum juice is applied for ethanol fermentation.

Cultivation of yeast strains in various sweet-stalk sorghum juice.

The possibility that the sweet-stalk sorghum juice may inhibit the growth of the yeast strain should be elucidated before being applied as fermentation media. Gibbons and Westby (1989) reported some factor(s) present in the juice from sweet sorghum cultivar NK 8368 inhibited the yeast growth. To test this possibility, eight yeast strains were grown aerobically in three kind of sweet-stalk sorghum juice based media and YEPD broth as a standard growth media. The resulted growth curves were presented in Figure 1.

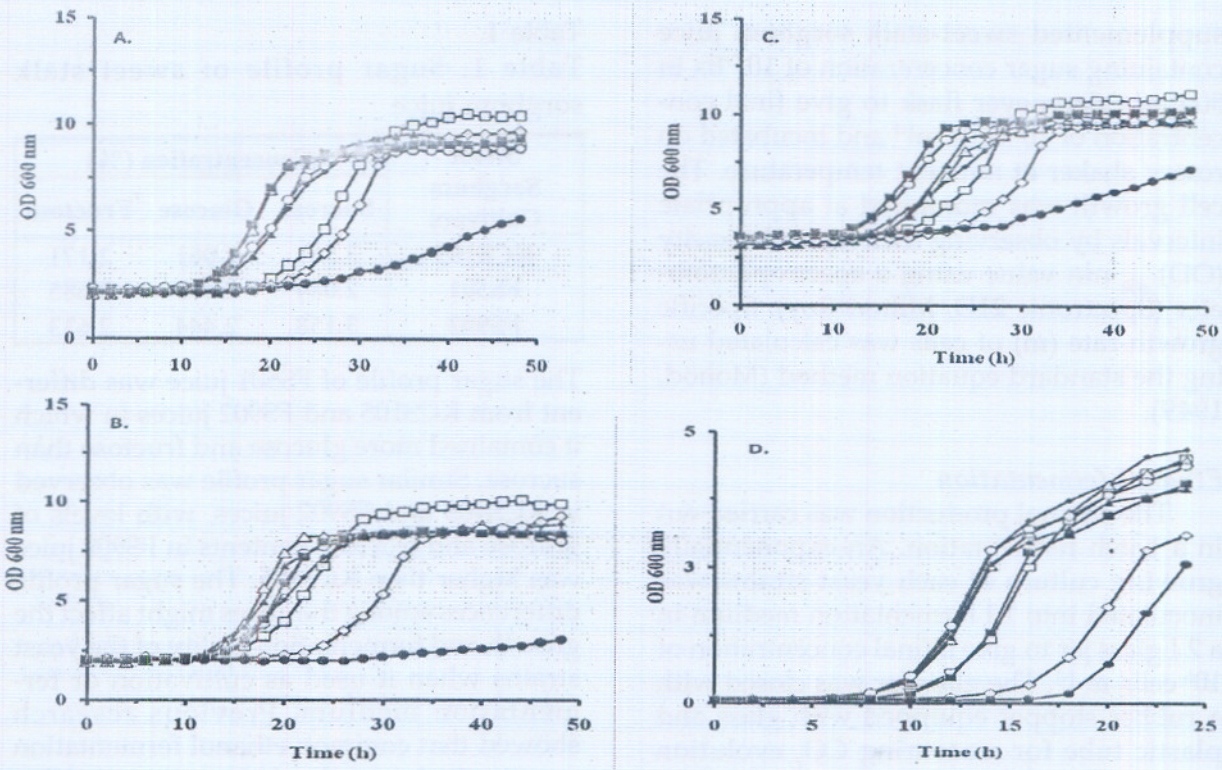


Figure 1. Cell growth of the yeast strains in cultivation media. Eight yeast strain: OUT7055 (◇), OUT7903 (■), OUT7921 (○), OUT7009 (◆), OUT7027 (□), OUT7913 (△), OUT7080 (●), and OUT7096 (▲) were grown in the sweet-stalk sorghum juices of KCS105 (A), FS501 (B), FS902 (C), and YEPD(D) medium. The cultures were shaken at ambient temperature and the cell growth was monitored by reading $OD_{600\ nm}$, as described above.

The growth profile of all yeast strains in the juice based medium were similar with that in the standard medium. The lag phase of all yeast strains required similar time whether they were grown in juice based media or in a standard medium. This finding indicated that the sweet-stalk sorghum juices did not contain any substance at a level that inhibit the growth of the yeast cells. Among eight yeast strains, the OUT7027 strain performed the highest cell density in all media tested, thus this strain might be the most potential candidate to be used in producing baker's yeast using sweet-stalk sorghum juices.

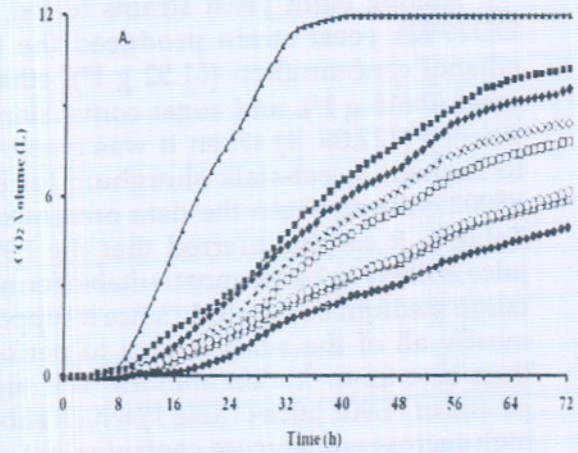
The specific growth rates (m) of all yeast strains in all media were presented in Table 2.

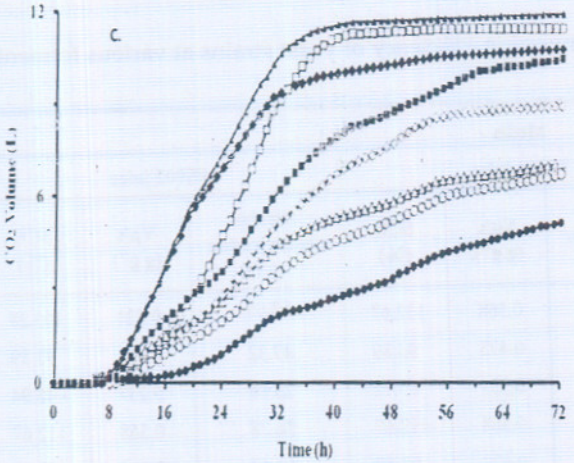
Table 2. Specific growth rate (m) of yeast strains at various cultivation media

Yeast strains	Media			
	YEPD	KCS105 juice	FS501 juice	FS902 juice
	Specific growth rate (h^{-1})			
OUT7903	0.730	0.863	0.955	0.808
OUT7055	0.492	0.931	0.951	0.980
OUT7096	0.673	0.805	0.901	0.669
OUT7913	0.675	0.956	0.894	0.626
OUT7921	0.686	0.660	0.880	0.621
OUT7027	0.663	0.767	0.730	1.053
OUT7009	0.711	0.476	0.480	0.380
OUT7080	0.457	0.159	0.072	0.145
Mean	0.636	0.702	0.733	0.660

Most of the yeast strains, except OUT7009 and OUT7080, had a better specific growth rates in sweet-stalk sorghum juice medium than in YEPD broth, and

among cultivation media tested in this experiment it is likely that sweet-stalk sorghum juice of FS501 was the best cultivation medium. The best specific growth rate of the yeast strains cultivated in the YEPD broth and the sweet-stalk sorghum juices of KCS105, FS501, and FS902 cultivars, were achieved by OUT7903 (0.730 h^{-1}, OUT7913 (0.956 h^{-1}), OUT7903 (0.955 h^{-1}), and OUT7027 (1.053 h^{-1}) yeast strains, respectively. The results suggested that the sweet-stalk sorghum juices could support the growth of the yeast strains.





Ethanol production of yeast strains in various sweet-stalk sorghum juices.

Progress of fermentation can be monitored visually by observing the rate of carbon dioxide (CO_2) evolution (Brown *et al.*, 1981; Sato and Yoshikawa, 1988). In this experiment the production of carbon dioxide was followed by observing the volume of CO_2 evolved periodically during fermentation and the accumulated volume of CO_2 was plotted as a fermentation curve. Curves describing the evolution of CO_2 by yeast cells fermenting sweet-stalk juices of various sweet sorghums were presented in Figure 2.

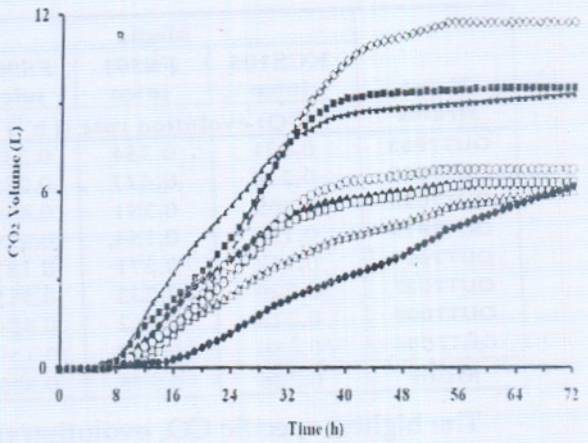


Figure 2. CO_2 Evolution Rate of yeast strains at various fermentation media. Eight yeast strain: OUT7055 (◇), OUT7903 (■), OUT7921 (○), OUT7009 (◆), OUT7027 (□), OUT7913 (△), OUT7080 (●), and OUT7096 (▲) were used to ferment the sweet-stalk sorghum juices of KCS105 (A), FS501 (B), and FS902 (C). The fermentation were done statically at ambient temperature and the CO_2 evolution was monitored by measuring the volume of CO_2 as described above.

It is noticeable that the amount of CO_2 produced by yeast strains varied depending upon juice medium. For example, the yeast strain OUT7027 performed a better fermentation only in sweet-stalk juice of FS902 cultivar which is in accordance with its growth in the same medium. Among the yeast strains, OUT7096 produced the high-

est amount of CO_2 in sweet-stalk juice of KCS105 and FS902 cultivars but not FS501.

The sweet-stalk juice of FS501 was observed to support strain OUT7055 producing the highest amount of CO_2.

Fermentation rate can be monitored as the rate of CO_2 evolution (Brown *et al.*, 1981). In this experiment the rate of CO_2 evolution was calculated using the amount of CO_2 produced during logarithmic phase and the results are presented in Table 3.

Table 3. Specific CO_2 evolution rate of yeast strains at various fermentation

Yeast strains	Media		
	KCS105 juice	FS501 juice	FS902 juice
	CO_2 evolution rate (l h^{-1})		
OUT7903	0.271	0.354	0.339
OUT7055	0.218	0.477	0.285
OUT7096	0.469	0.381	0.466
OUT7913	0.128	0.154	0.229
OUT7921	0.112	0.271	0.187
OUT7027	0.229	0.233	0.515
OUT7009	0.223	0.212	0.484
OUT7080	0.156	0.147	0.157
Mean	0.226	0.278	0.333

The highest specific CO_2 evolution rate of the yeast strains during fermentation of the juices extracted from KCS105, FS501, and FS902 sweet sorghum cultivars, were achieved by OUT7096 (0.469 l h^{-1}), OUT7055 (0.477 l h^{-1}), and OUT7027 (0.515 l h^{-1}), respectively. Based on these results and the average of yeast specific CO_2 evolution rates in each medium, it is likely that the sweet-stalk sorghum juice of FS902 was the most suitable fermentation medium.

The ethanol concentration, ethanol yield, and sugar conversion efficiency (SCE) for the eight strains studied are shown in Table 4

Among eight yeast strains tested, the OUT7921 yeast strain produced the best ethanol concentration (61.52 g l^{-1}), ethanol yield (0.615 g l^{-1}), and sugar conversion efficiency (122.06 %) when it was employed to ferment sweet-stalk shorghum juice of FS902 cultivar. From the data presented in Table 4, it can be inferred that the FS902 juice is likely to be the most suitable fermentation medium in this study since it supports mostly all of the yeast strains to perform their best fermentation abilities. The sugar profile of FS902 juice (Table 1) which shows high sucrose and glucose content might correlate with this result since yeast *saccharomyces cerevisiae* prefer to ferment those sug-

Table 4. Ethanol Concentration, Ethanol Yield, Sugar Conversion Efficiency of yeast strains at various fermentation media.

Yeast strains	Media								
	KCS105 juice			FS501 juice			FS902 juice		
	Alcohol concentration (g/l)	Yp/s (g g^{-1})	SCE (%)	Alcohol concentration (g/l)	Yp/s (g g^{-1})	SCE (%)	Alcohol concentration (g/l)	Yp/s (g g^{-1})	SCE (%)
OUT7903	37.86	0.379	75.11	56.78	0.568	112.67	52.05	0.521	103.28
OUT7055	47.32	0.473	93.89	47.32	0.473	93.89	47.32	0.473	93.89
OUT7096	33.12	0.331	65.72	33.12	0.331	65.72	23.66	0.237	45.94
OUT7913	47.32	0.473	93.89	56.78	0.568	112.67	56.78	0.568	112.67
OUT7921	47.32	0.473	93.89	47.32	0.473	93.89	61.52	0.615	122.06
OUT7027	23.66	0.237	46.94	23.66	0.237	46.94	52.05	0.521	103.28
OUT7009	42.59	0.426	84.50	33.12	0.331	65.72	28.39	0.284	56.33
OUT7080	4.73	0.047	9.39	9.46	0.095	18.78	23.66	0.237	46.94
Mean	35.49	0.355	70.42	38.45	0.384	76.29	43.18	0.432	85.55

ars (Berthels *et al.*, 2004; Imam and Capareda, 2010; Meneses *et al.*, 2002) and leave the fructose as major residual sugar in the fermentation medium (Wu *et al.*, 2010).

A yeast strain which has a sugar conversion efficiency (SCE) greater than 90% can be used to improve ethanol yields from sweet sorghum juice (De Mancilha *et al.*, 1984). In this study five yeast strains, OUT7093, OUT7055, OUT7913, OUT7921, and OUT7027, have a SCE greater than 90%. However, only three yeast strains, OUT7055, OUT7913, and OUT7921, consistently have SCE greater than 90% in sweet-stalk sorghum juice fermentation media. Thus, they are promising yeast strains which can be used for improving ethanol yields from sweet sorghum juice.

Acknowledgements

We thank Youji Nitta of Ibaraki University for sweet sorghum cultivars and Yoshinobu Kaneko of Osaka University for yeast strains.

References

Berthels, N.J., R.R.C. Otero, F.F. Bauer, J.M. Thevelein, and I.S. Pretorius. 2004. Discrepancy in glucose and fructose utilization during fermentation by *Saccharomyces cerevisiae* wine yeast. FEMS *Yeast Res.* **4**, 683–689

Brown, S.W., S. G. Oliver, D. E. F. Harrison, and R. C. Righelato. 1981. Ethanol inhibition of yeast growth and fermentation: differences in the magnitude and complexity of the effect. *European J. Appl. Microbiol. Biotechnol.* **11**, 151–155.

Day, D.F. and D.Sarkar. 1982. Fuel alcohol from sweet sorghum : microbial aspects. Dev. Ind. Microbiol. **23**, 361–366.

De Mancilha, I.M., A.M. Pearson, J. Waller, and G.J. Hogaboam. 1984. Increasing alcohol yield by selected yeast fermentation of sweet sorghum. I. Evaluation of yeast strains for ethanol production. Biotechnol. Bioeng. **26** (6), 632–634.

Gibbons, R.W. and A.C. Westby. 1989. Cofermentation of sweet sorghum juice and grain for production of fuel ethanol and distillers wet grain. *Biomass.* **18**, 43–57.

Gnansounou, E. and A. Dauriat. 2005. Ethanol fuel from biomass : A review. *J. Sci. Ind. Res.* **64**, 809 – 821.

Imam, T. and S. Capareda. 2010. Ethanol fermentation from sweet sorghum juice. ASABE Paper No. 1009693. St. Joseph Mich.:ASABE.

Kaye, S. 1980. A rapid screening blood alcohol analysis for the local pathologist. *Am. J. For. Med. and Path.* **1**, 205–208.

Laopaiboon, L., P. Thanonkeo, P. Jaisil, and P.Laopaiboon. 2007 Ethanol production from sweet sorghum juice in batch and fed-batch fermentations by *Saccharomyces cerevisiae*. *World J. Microbiol. Biotechnol.* **23**, 1497–1501.

Liu, R., J. Li, and F. Shen. 2008. Refining bioethanol from stalk juice of sweet sorghum by immobilized yeast fermentation. *Renew. Energy*, **33**, 1130–1135.

Meneses, F.J., P.A. Henschke, and V. Jiranek. 2002. A survey of industrial strains of *Saccharomyces cerevisiae* reveals numerous altered patterns of maltose and sucrose utilization. *J. Inst. Brew.* **108**, 310–321.

Monod, J. 1949. The growth of bacterial cultures. *Annu. Rev. Microbiol.* **3**, 371–394

Nitta, Y., A. Kamiyama, T. Matsuda, S. Nakamura, Y. Goto, E. Inoue, K. Narisawa, Y. Kurusu, H. Ohta, S. Chonan, A. Toyoda, T. Kato, H. Kobayashi, M. Komatsuzaki, and T.

Sato. 2008. Sweet sorghum cultivation as bio-fuel crop in Ibaraki prefecture. *J. Crop Sci.* (Extra1), 90–91.

Prasad, S., A. Singh, N. Jain, and H.C. Hoshi. 2007. Ethanol production from sweet sorghum syrup for utilization as automotive fuel in India. *Energy Fuels* **21**, 2415–2420.

Rooney, W.L., J. Blumenthal, B. Bean, and J.E. Mullet. 2007. Designing sorghum as a dedicated bioenergy feedstock. *Biofuels Bioprod. Bioref.* **1**, 147–157.

Sato, K. and K. Yoshikawa.1988. Growth and growth estimation of Saccharomyces cerevisiae in solid-state ethanol fermentation. *J. Ferment. Technol.* **66**, 667–673.

Saxena, R.C., D.K. Adhikari, and H.B. Goyal. 2009. Biomass-based energy fuel through biochemical routes: A review. Renewable and Sustainable Energy Reviews. 13, 167–178.

Steduto, P., N. Katerji, and H. Puertos-Molina. 1997. Water-use efficiency of sweet sorghum under water stress conditions: gas-exchange investigations at leaf and canopy scales. *Field Crop Res.* **54**, 221–234.

Sumantri, A. and E. Purnomo. 1997. Sweet sorghum research and development in Indonesia. Proc. 1st Int. Sweet Sorghum Conf., Inst. Bot. Chin. Acad. Sci., China. 49–57.

Tsuchihashi, N. and Y. Goto. 2004. Cultivation of sweet sorghum (*Sorghum bicolor* (L.) Moench) and determination of its harvest time to make use as the raw material for fermentation, practiced during rainy season in dry land of Indonesia. *Plant Prod. Sci.* **7**, 442 – 448.

Tsuchihashi, N. and Y. Goto. 2005. Internode characteristics of sweet sorghum (*Sorghum bicolor* (L.) Moench) during dry and rainy seasons in Indonesia. *Plant Prod. S* 607.

Wu, X., S. Staggenborg, J. L. Pr L. Rooney, J. Yu, and D. Features of sweet sorghu their performance i fermentation. *Industria Products.* **31**, 164–170